Effects of neem seed extract on the growth of fungal associates of the mountain pine beetle

R.J. NEVILL and L. SAFRANYIK

CANADIAN FOREST SERVICE, PACIFIC FORESTRY CENTRE
506 W. BURNSIDE RD., VICTORIA, BC V8Z 1M5

ABSTRACT

We investigated the fungicidal activity of neem seed extract, active ingredient azadirachtin, to *Ophiostoma clavigerum* (Robins. Jeff. & Davids) and *Ophiostoma ips* (Rumb.), the fungal associates of the mountain pine beetle, *Dendroctonus ponderosae* Hopk. When added to malt extract agar, concentrations of 100 ppm significantly reduced the growth of both fungal associates and concentrations above 250 ppm killed both fungi.

Key words: Azadirachtin, bluestain fungi, Coleoptera, Scolytidae

DISCUSSION

Mortality caused by the mountain pine beetle, *Dendroctonus ponderosae* Hopk., results in losses of millions of cubic metres of mature pines annually in British Columbia (Unger 1993). Present management techniques to reduce mountain pine beetle attack include sanitation logging of large infested areas, single-tree treatments, and the use of trap trees baited with pheromones (Unger 1993, Safranyik 1995). Single-tree treatments and baited-tree treatments usually consist of wintertime "fall and burn" or injection of a systemic pesticide, monosodium methane arsenate (MSMA). However, concern arises because this pesticide is potentially toxic to applicators and other organisms (MacLauchlin *et al.* 1988).

A potentially new control method for managing the mountain pine beetle is the use of neem seed extracts from the neem tree, *Azadirachta indica* A. Juss (Naumann *et al.* 1994). Neem seed extracts have several useful qualities for pest management including insecticidal and fungitoxic properties as well as low mammalian toxicity (Narasimhan *et al.* 1993, Koul *et al.* 1990, Schmutterer 1990).

The insecticidal activity of neem seed extracts on brood development of the mountain pine beetle have already been investigated (Naumann *et al.* 1994), but the question remained whether this compound was also active against *Ophiostoma clavigerum* (Robins. Jeff. & Davids) and *Ophiostoma ips* (Rumb.), the fungal associates of the mountain pine beetle (Robinson 1962, Reid *et al.* 1967, Whitney 1971).

Inoculation studies show these fungi can kill trees in the absence of their bark beetle vector (references in Owen *et al.* 1987) and these fungi also cause a reduction in the value of timber or timber products by discoloring sapwood (Behrendt *et al.* 1995). Therefore, fungicidal activity of neem seed extracts against bluestain fungi would provide an additional benefit while controling the mountain pine beetle.

To determine the fungicidal properties of neem seed extracts to *O. ips* and *O. clavigerum,* a proprietary emulsifiable concentrate of the extracts containing 5% active ingredient (azadirachtin) was obtained from Phero Tech Inc. (Delta, B.C., Canada). Dilutions were made with ethanol and added to malt extract agar (MEA) at rates expressed in terms of ppm of azadirachtin. *Ophiostoma clavigerum* and *O. ips* cultures were grown for 2 weeks on individual Petri plates of plain MEA. At this time 5 mm MEA

plugs colonized with either fungus were transferred to separate Petri dishes containing MEA with 0, 1, 10, 100, 250, and 500 ppm azadirachtin. Growth of the fungi across the plates was measured daily. Daily growth was determined for 3 replications of 3 Petri plates per concentration per fungus and observed over 28 days. Data were analyzed by a one-way ANOVA and by subsequent Student Neuman-Keuls tests (SAS 1989). Azadirachtin began showing significant fungicidal activity to *O. ips* at 10 ppm and growth of both fungi was significantly inhibited at 100 ppm (Table 1). No growth occurred at 250 or 500 ppm over the 28-day period and when the original plugs were transferred to new MEA without azadirachtin, no growth occurred.

Table 1.

Growth rate (mm/day) of *Ophiostoma ips* and *O. clavigerum* after 28 days on malt extract agar with different concentrations of neem seed extract, azadirachtin

	azadirachtin /ppm					
Fungus	Control	1	10	100	250	500
O.ips	7.80a[1]	7.75a	4.90b	0.63c	0.0d	0.0d
O.Clavigerum	4.87a	4.87a	4.74a	0.25b	0.0c	0.0c

[1] treatments in the same row followed by the same letter are not significantly different ($p \geq 0.05$, GLM procedure and Student Neuman-Keuls test).

Combined with its insecticidal properties, this compound may prove to be an effective control for both the mountain pine beetle and its fungal associates.

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